(Abstract)

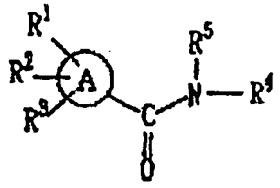
(Amended)

Object

To put forward a nitric oxide synthase inhibitor.

Method of Solution

The nitric oxide synthase inhibitor containing as effective ingredient amide derivative represented by general formula

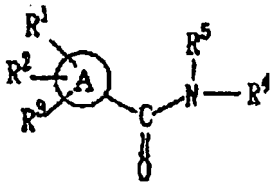


As the aforesaid amide derivative, for example N-(2-n-butyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl)-2,3,4-trimethoxy benzamide, N-(2-n-butyl-9H-purine-6-yl)-3,4,5-trimethoxy benzamide, N-(2-n-butyl thieno[3,2-d]pyrimidine-4-yl)-3,4,5-trimethoxy benzamide.

Patent Claims

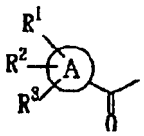
Claim 1

A nitric oxide synthase inhibitor characterised by containing effective dose of amide derivative represented by general formula as effective ingredient together with preparation carrier



[wherein, a ring represented by A is benzene ring, naphthalene ring, pyridine ring or furan ring, and when A is other than benzene ring, all R1, R2 and R3 denote hydrogen atoms and when A is benzene ring, they may be the same or different and denote hydrogen atom, lower alkoxy group, halogen atom, nitro group, lower alkyl group, halogen substituted lower alkyl group, phenyl group, phenoxy group, lower alkanoyloxy group, hydroxy group, lower alkyl thio group, lower alkyl sulfinyl group or lower alkyl sulphonyl group. R4 denotes the heterocyclic group selected from (1) thieno[3,2-d]pyrimidin-4-yl group having lower alkyl group as substituent, (2) pyrazolo [1,5-a]-1,3,5-triazin-4-yl group optionally containing 1-2 groups selected from the lower alkyl group, phenyl group, phenyl lower alkyl group, phenylthio phenyl group and halogen atom as substituent, (3) pyrazolo[3,4-d]pyrimidin-4-yl group containing lower alkyl group at 6-position and moreover in which one of nitrogen atom is optionally-substituted by phenyl lower alkyl

group and (4) purin-6-yl group containing lower alkyl group at 2-position and moreover in which one of nitrogen atom is optionally-substituted by lower alkyl group or phenyl lower alkyl group, and R5 denotes hydrogen atom or a group



(wherein, A, R1, R2 and R3 are same as above)].

Claim 2

A nitric oxide synthase inhibitor in accordance with Claim 1 containing as an effective ingredient the compound wherein in the general formula in accordance with Claim 1, A is benzene ring and R5 is hydrogen atom.

Claim 3

A nitric oxide synthase inhibitor in accordance with Claim 2 containing as an effective ingredient the compound wherein in the general formula in accordance with Claim 1, heterocyclic group represented by R4 is thieno[3,2-d]pyrimidin-4-yl group containing lower alkyl group at 2-position as substituent, pyrazolo[1,5-a]-1,3,5-triazin-4-yl group containing lower alkyl group at 2-position as substituent or 9H-purin-6-yl group containing lower alkyl group at 2-position.

Claim 4

A nitric oxide synthase inhibitor in accordance with Claim 3 containing as an effective ingredient the compound wherein in general formula in accordance with Claim 1, R1, R2 and R3 are the same or different and are hydrogen atoms, lower alkoxy groups or halogen atoms.

Claim 5

A nitric oxide synthase inhibitor in accordance with Claim 4 containing as an effective ingredient at least one species of compound selected from the N-(2-n-butyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl)-2,3,4-trimethoxy benzamide, N-(2-n-butyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl)-2-methoxy benzamide, N-(2-n-butyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl)-2,4-dichlorobenzamide, N-(2-n-butyl-9H-purine-6-yl)-3,4,5-trimethoxy benzamide, N-(2-n-butyl thieno[3,2-d]pyrimidine-4-yl)-3,4,5-trimethoxy benzamide, N-(2-n-butyl thieno[3,2-d]pyrimidine-4-yl)-2-chlorobenzamide, N-(2-n-butyl thieno[3,2-d]pyrimidine-4-yl)-2,4-dichlorobenzamide and N-(2-n-butyl thieno[3,2-d] pyrimidine-4-yl)-3-methoxy benzamide.

Claim 6

A nitric oxide synthase inhibitor in accordance with Claim 5, wherein the effective ingredient is N-(2-n-butyl thieno[3,2-d]pyrimidine-4-yl)-3,4,5-trimethoxy benzamide.

Claim 7

A nitric oxide synthase inhibitor in accordance with any of Claims 1-6 which inhibits inducible-type nitric oxide synthase selectively.

Claim 8

Prevention and treatment agent of septicaemia characterised by containing effective dose of the amide derivatives in accordance with Claim 1 together with a non-toxic carrier.

Claim 9

The endotoxin shock improvement agent characterised by containing effective dose of the amide derivatives in accordance with Claim 1 together with a non-toxic carrier.

Detailed Description of the Invention

(0001)

Technical Sphere of the Invention

This invention relates to the following, namely, a novel nitric oxide synthase inhibitor, more particularly a drug which inhibits the induction of inducible-type nitric oxide synthase.

(0002)

Technology of the Prior Art

In the first half of 1980's, it was discovered for the first time during the study of nitroxide in vivo that nitric oxide (hererinafter called NO) was produced in vivo. Since this discovery, NO attracted attension of many researchers, and it was reported in 1987 that the NO was the main body of vascular endothelium derived relaxing factor. Moreover, presently, physiological function of NO and relation to pathology have been made clear in many fields such as cardiovascular, immunity, nervous system.

(0003)

One of them is the fact that the NO constantly produced in-vivo has been elucidated to play an important role in maintenance of homeostasis of cardiovascular dynamics. Moreover, in septicemia, large quantity of NO is produced from the cytokine activated by endotoxin and this is said to cause endotoxic shock state such as endothelial cell disorder, myocardium contractive force lowering or the like.

(0004)

NO is produced from the L-arginine by NO synthase (hereinafter called NOS). The said enzyme can be classified broadly to inducible NOS (hereinafter called iNOS) linked with pathological NO production and constitutive NOS (hereinafter called cNOS) which is always expressed.

(0005)

As described above, because NO participates in various kinds of diseases such as septicemia or the like, research has been carried out into the elucidation of the mechanism thereof and eventually towards an NOS inhibitor for the purpose of application as therapeutic drug of these diseases. As representative example of the said inhibitor, arginine analogue such as N-omega-nitro-L-arginine and the like may be proposed.

(0006)

However, most of the NOS inhibitors familiar to the prior art including the aforesaid representative example inhibit cNOS in addition iNOS, and as a result of the use of these as therapeutic agents, the control of homeostatic cardiovascular dynamics is also inhibited, and side effects such as elevation of blood pressure, organ blood flow decrease or the like cannot be avoided. Furthermore, during the use of these, problems such as effects on central nervous system, impotency and the like also give concern.

(0007)

In this way, the NOS inhibitors familiar to the prior art cannot be evaluated as pharmaceuticals, and there is a demand in this field for new substances which can selectively hinder iNOS instead of these.

(0008)

Problems to be Overcome by this Invention

Accordingly, the object of this invention is to put forward a substance which could selectively only hinder iNOS as desired in this field and also an NOS inhibitor using this.

(0009)

A study group of these inventors performed research and analysis into the synthesis of various kinds of compound and their pharmacologic actions, with the object of developing a drug preparation effective ingredient compound, and in that process, this group succeeded in the synthesis of a series of amide derivatives having strong analgesia action, and invention relating to such compounds sthese or the like was applied for (WO97/46560).

(0010)

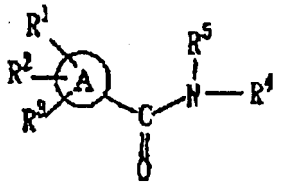
In subsequent investigations, these inventors, have made a new discovery, that the fact of aforesaid series of compounds have iNOS induction inhibitory action, separate from their analgesic action and moreover unrelated to that action, and in addition, markedly reduced side effects. This invention was completed based on this discovery here.

(0011)

Means to Overcome these Problems

In other words, in accordance with this invention, it is put forward NOS inhibitor containing the amide derivative represented by following general formula (1) as effective ingredient, together with the preparation carrier.

(0012)

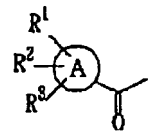


(1)

(0013)

[Wherein, a ring represented by A is benzene ring, naphthalene ring, pyridine ring or furan ring, and when A is other than benzene ring, all R1, R2 and R3 denote hydrogen atoms and when A is benzene ring, they may be the same or different and denote hydrogen atom, lower alkoxy group, halogen atom, nitro group, lower alkyl group, halogen substituted lower alkyl group, phenyl group, phenoxy group, lower alkanoyloxy group, hydroxy group, lower alkyl thio group, lower alkyl sulfinyl group or lower alkyl sulphonyl group. R4 denotes the heterocyclic group selected from (1) thieno[3,2-d]pyrimidin-4-yl group having lower alkyl group as substituent, (2) pyrazolo [1,5-a]-1,3,5-triazin-4-yl group optionally containing 1-2 groups selected from the lower alkyl group, phenyl group, phenyl lower alkyl group, phenylthio phenyl group and halogen atom as substituent, (3) pyrazolo[3,4-d]pyrimidin-4-yl group containing lower alkyl group at 6-position and moreover in which one of nitrogen atom is optionally-substituted by phenyl lower alkyl group and (4) purin-6-yl group containing lower alkyl group at 2-position and moreover in which one of nitrogen atom is optionally-substituted by lower alkyl group or phenyl lower alkyl group, and R5 denotes hydrogen atom or a group

(0014)



(0015)

(wherein, A, R1, R2 and R3 are same as above)].

The derivatives represented by the aforesaid general formula (1) have NOS inhibit action, in particular, the action to inhibit iNOS selectively. Accordingly, it is characterised by the point that it is almost free from side effects such as pressure increase, reduction of blood flow of organs, bad influence to central nervous system and the like.

(0016)

As each group in the aforesaid general formula (1) denoting the effective ingredient of NOS inhibitor of this invention, for example the following each group can be exemplified. Moreover, it is assumed that the term "lower" denotes carbon number 1-6 in each group.

(0017)

As lower alkyl group, straight or branched chain lower alkyl group such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, hexyl group and the like can be exemplified.

(0018)

As lower alkoxy group, methoxy, ethoxy, propoxy, isopropoxy, butoxy, pentyloxy, hexyloxy groups can be exemplified.

(0019)

As phenyl lower alkyl group, benzyl, 1-phenylethyl, 2-phenylethyl, 3-phenylpropyl, 4-phenylbutyl, 5-phenyl pentyl, 6-phenylhexyl group can be exemplified.

(0020)

As halogen atom, fluorine atom, chlorine atom, bromine atom and iodine atom are included.

(0021)

As halogen substituted lower alkyl group, trifluoromethyl, pentafluoro ethyl, heptafluoro propyl, nonafluoro butyl, undeca fluoro pentyl, trideca fluoro hexyl group can be exemplified.

(0022)

As lower alkanoyloxy group, acetoxy, propionyloxy, butyryl oxy, valeryl oxy, hexanoyloxy, heptanoyloxy group can be exemplified.

(0023)

As lower alkyl thio group, methylthio, ethylthio, propylthio, butylthio, pentyl thio, hexyl thio group can be exemplified.

(0024)

As lower alkyl sulfinyl group, methylsulfinyl, ethyl sulphinyl, propylsulphinyl, butyl sulphinyl, pentyl sulphinyl, hexyl sulfinyl group can be exemplified.

(0025)

As lower alkyl sulphonyl group, methylsulfonyl, ethylsulfonyl, propyl sulfonyl, butylsulfonyl, pentyl sulfonyl, hexyl sulphonyl group can be exemplified.

(0026)

In heterocyclic group represented by R4, as thieno[3,2-d]pyrimidin-4-yl containing lower alkyl group as substituent shown in (1), 2-methyl thieno[3,2-d]pyrimidine-4-yl, 2-ethyl thieno[3,2-d] pyrimidine-4-yl, 2-n-propyl thieno[3,2-d]pyrimidine-4-yl, 2-n-butyl thieno[3,2-d]pyrimidine-4-yl, 2-n-pentyl thieno[3,2-d]pyrimidine-4-yl, 2-n-hexyl thieno[3,2-d]pyrimidin-4-yl group can be exemplified.

(0027)

In heterocyclic group represented by R4, as pyrazolo[1,5-a]-1,3,5-triazin-4-yl group optionally containing 1-2 groups selected from the lower alkyl group, phenyl, phenyl lower alkyl group, phenylthio phenyl group and halogen atom as substituent shown in (2), in addition to the unsubstituted group, following each substituted pyrazolo[1,5-a]-1,3,5-triazin-4-yl group can be exemplified.

(0028)

2-methylpyrazolo[1,5-a]-1,3,5-triazine-4-yl, 2-ethyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl, 2-n-propyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl, 2-n-butyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl, 2-n-pentyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl, 2-n-hexyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl, 2-phenylpyrazoro[1,5-a]-1,3,5-triazine-4-yl, 2-benzyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl, 2-(2-phenylethyl) pyrazolo[1,5-a]-1,3,5-triazine-4-yl, 2-(3-phenylpropyl) pyrazolo[1,5-a]-1,3,5-triazine-4-yl, 2-(4-phenylbutyl) pyrazolo[1,5-a]-1,3,5-triazine-4-yl, 2-(5-phenyl pentyl) pyrazolo [1,5-a]-1,3,5-triazine-4-yl, 2-(6-phenylhexyl) pyrazolo[1,5-a]-1,3,5-triazine-4-yl, 2-methyl-8-phenylpyrazoro[1,5-a]-1,3,5-triazine-4-yl, 2-ethyl-8-phenylpyrazoro[1,5-a]-1,3,5-triazine-4-yl, 8-phenyl-2-n-propyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl, 2-n-butyl-8-phenylpyrazoro[1,5-a]-1,3,5-triazine-4-yl, 2-n-pentyl-8-phenylpyrazoro[1,5-a]-1,3,5-triazine-4-yl, 2-n-hexyl-8-phenylpyrazoro[1,5-a]-1,3,5-triazine-4-yl, 2-methyl-7-phenylpyrazoro[1,5-a]-1,3,5-triazine-4-yl, 2-ethyl-7-phenylpyrazoro[1,5-a]-1,3,5-triazine-4-yl, 7-phenyl-2-n-propyl pyrazolo[1,5-a]-1,3,5-

triazine-4-yl, 2-n-butyl-7-phenylpyrazoro[1,5-a]-1,3,5-triazine-4-yl, 2-n-pentyl-7-phenylpyrazoro [1,5-a]-1,3,5-triazine-4-yl, 2-n-hexyl-7-phenylpyrazoro[1,5-a]-1,3,5-triazine-4-yl, 2-methyl-8-(4-phenylthio phenyl) pyrazolo[1,5-a]-1,3,5-triazine-4-yl, 2-ethyl-8-(4-phenylthio phenyl) pyrazolo [1,5-a]-1,3,5-triazine-4-yl, 8-(4-phenylthio phenyl)-2-n-propyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl, 2-n-butyl-8-(4-phenylthio phenyl) pyrazolo[1,5-a]-1,3,5-triazine-4-yl, 2-n-pentyl-8-(4-phenylthio phenyl) pyrazolo[1,5-a]-1,3,5-triazine-4-yl, 2-n-hexyl-8-(4-phenylthio phenyl) pyrazolo[1,5-a]-1,3,5-triazine-4-yl, 8-bromo-2-methylpyrazolo[1,5-a]-1,3,5-triazine-4-yl, 8-bromo-2-ethyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl, 8-bromo-2-n-propyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl, 8-bromo-2-n-butyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl, 8-bromo-2-n-pentyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl, 8-bromo-2-n-hexyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl, 2-n-butyl-8-fluoro pyrazolo[1,5-a]-1,3,5-triazine-4-yl, 2-n-butyl-8-chloro pyrazolo[1,5-a]-1,3,5-triazine-4-yl, 2-n-butyl-8-iodo pyrazolo[1,5-a]-1,3,5-triazin-4-yl group and the like.

(0029)

In the heterocyclic group represented by R4, as pyrazolo[3,4-d]pyrimidin-4-yl group containing lower alkyl group at 6-position and further in which one of nitrogen atom is optionally-substituted by phenyl lower alkyl group which is shown in (3), the following each group can be exemplified.

(0030)

6-methyl-1H-pyrazolo[3,4-d]pyrimidine-4-yl, 6-ethyl-1H-pyrazolo[3,4-d]pyrimidine-4-yl, 6-n-propyl-1H-pyrazolo[3,4-d]pyrimidine-4-yl, 6-n-butyl-1H-pyrazolo[3,4-d]pyrimidine-4-yl, 6-n-pentyl-1H-pyrazolo[3,4-d]pyrimidine-4-yl, 6-n-hexyl-1H-pyrazolo[3,4-d]pyrimidine-4-yl, 6-methyl-2H-pyrazolo[3,4-d]pyrimidine-4-yl, 6-ethyl-2H-pyrazolo[3,4-d]pyrimidine-4-yl, 6-n-propyl-2H-pyrazolo[3,4-d]pyrimidine-4-yl, 6-n-butyl-2H-pyrazolo[3,4-d]pyrimidine-4-yl, 6-n-pentyl-2H-pyrazolo[3,4-d]pyrimidine-4-yl, 6-n-hexyl-2H-pyrazolo[3,4-d]pyrimidine-4-yl, 2-benzyl-6-methyl-2H-pyrazolo[3,4-d]pyrimidine-4-yl, 2-benzyl-6-ethyl-2H-pyrazolo[3,4-d] pyrimidine-4-yl, 2-benzyl-6-n-propyl-2H-pyrazolo[3,4-d]pyrimidine-4-yl, 2-benzyl-6-n-butyl-2H-pyrazolo[3,4-d]pyrimidine-4-yl, 2-benzyl-6-n-pentyl-2H-pyrazolo[3,4-d]pyrimidine-4-yl, 2-benzyl-6-n-hexyl-2H-pyrazolo[3,4-d]pyrimidine-4-yl, 6-n-butyl-2-(1-phenylethyl)-2H-pyrazolo [3,4-d]pyrimidine-4-yl, 6-n-butyl-2-(2-phenylethyl)-2H-pyrazolo[3,4-d]pyrimidine-4-yl, 6-n-butyl-2-(3-phenylpropyl)-2H-pyrazolo[3,4-d]pyrimidine-4-yl, 6-n-butyl-2-(4-phenylbutyl)-2H-pyrazolo[3,4-d]pyrimidine-4-yl, 6-n-butyl-2-(5-phenyl pentyl)-2H-pyrazolo[3,4-d]pyrimidine-4-yl, 6-n-butyl-2-(6-phenylhexyl)-2H-pyrazolo[3,4-d]pyrimidine-4-yl, 1-benzyl-6-methyl-1H-pyrazolo[3,4-d]pyrimidine-4-yl, 1-benzyl-6-ethyl-1H-pyrazolo[3,4-d]pyrimidine-4-yl, 1-benzyl-6-n-propyl-1H-pyrazolo[3,4-d]pyrimidine-4-yl, 1-benzyl-6-n-butyl-1H-pyrazolo[3,4-d] pyrimidine-4-yl, 1-benzyl-6-n-pentyl-1H-pyrazolo[3,4-d]pyrimidine-4-yl, 1-benzyl-6-n-hexyl-1H-pyrazolo[3,4-d]pyrimidine-4-yl, 6-n-butyl-1-(1-phenylethyl)-1H-pyrazolo[3,4-d]pyrimidine-

4-yl, 6-n-butyl-1-(2-phenylethyl)-1H-pyrazolo[3,4-d]pyrimidine-4-yl, 6-n-butyl-1-(3-phenylpropyl)-1H-pyrazolo[3,4-d]pyrimidine-4-yl, 6-n-butyl-1-(4-phenylbutyl)-1H-pyrazolo [3,4-d]pyrimidine-4-yl, 6-n-butyl-1-(5-phenyl pentyl)-1H-pyrazolo[3,4-d]pyrimidine-4-yl, 6-n-butyl-1-(6-phenylhexyl)-1H-pyrazolo[3,4-d]pyrimidine-4-yl, 6-n-butyl-5H-pyrazolo[3,4-d] pyrimidine-4-yl, 5-benzyl-6-n-butyl-5H-pyrazolo[3,4-d]pyrimidin-4-yl group and the like.

(0031)

Moreover, in heterocyclic group represented by R4, as purin-6-yl group containing lower alkyl group at 2-position and further in which one of nitrogen atoms is optionally-substituted by lower alkyl group or phenyl lower alkyl group which is shown in (4), the following each group can be exemplified.

(0032)

2-methyl-9H-purine-6-yl, 2-ethyl-9H-purine-6-yl, 2-n-propyl-9H-purine-6-yl, 2-n-butyl-9H-purine-6-yl, 2-n-pentyl-9H-purine-6-yl, 2-n-hexyl-9H-purine-6-yl, 2-methyl-7H-purine-6-yl, 2-ethyl-7H-purine-6-yl, 2-n-propyl-7H-purine-6-yl, 2-n-butyl-7H-purine-6-yl, 2-n-pentyl-7H-purine-6-yl, 2-n-hexyl-7H-purine-6-yl, 9-benzyl-2-methyl-9H-purine-6-yl, 9-benzyl-2-ethyl-9H-purine-6-yl, 9-benzyl-2-n-propyl-9H-purine-6-yl, 9-benzyl-2-n-butyl-9H-purine-6-yl, 9-benzyl-2-n-pentyl-9H-purine-6-yl, 9-benzyl-2-n-hexyl-9H-purine-6-yl, 2-n-butyl-9-(1-phenylethyl)-9H-purine-6-yl, 2-n-butyl-9-(2-phenylethyl)-9H-purine-6-yl, 2-n-butyl-9-(3-phenylpropyl)-9H-purine-6-yl, 2-n-butyl-9-(4-phenylbutyl)-9H-purine-6-yl, 2-n-butyl-9-(5-phenyl pentyl)-9H-purine-6-yl, 2-n-butyl-9-(6-phenylhexyl)-9H-purine-6-yl, 2,9-dimethyl-9H-purine-6-yl, 2-ethyl-9-methyl-9H-purine-6-yl, 9-methyl-2-n-propyl-9H-purine-6-yl, 2-n-butyl-9-methyl-9H-purine-6-yl, 9-methyl-2-n-pentyl-9H-purine-6-yl, 2-n-hexyl-9-methyl-9H-purine-6-yl, 7-benzyl-2-methyl-7H-purine-6-yl, 7-benzyl-2-ethyl-7H-purine-6-yl, 7-benzyl-2-n-propyl-7H-purine-6-yl, 7-benzyl-2-n-butyl-7H-purine-6-yl, 7-benzyl-2-n-pentyl-7H-purine-6-yl, 7-benzyl-2-n-hexyl-7H-purine-6-yl, 2-n-butyl-7-(1-phenylethyl)-7H-purine-6-yl, 2-n-butyl-7-(2-phenylethyl)-7H-purine-6-yl, 2-n-butyl-7-(3-phenylpropyl)-7H-purine-6-yl, 2-n-butyl-7-(4-phenylbutyl)-7H-purine-6-yl, 2-n-butyl-7-(5-phenyl pentyl)-7H-purine-6-yl, 2-n-butyl-7-(6-phenylhexyl)-7H-purine-6-yl, 2,7-dimethyl-7H-purine-6-yl, 2-ethyl-7-methyl-7H-purine-6-yl, 7-methyl-2-n-propyl-7H-purine-6-yl, 2-n-butyl-7-methyl-7H-purine-6-yl, 7-methyl-2-n-pentyl-7H-purine-6-yl, 2-n-hexyl-7-methyl-7H-purine-6-yl, 1-benzyl-2-methyl-1H-purine-6-yl, 1-benzyl-2-ethyl-1H-purine-6-yl, 1-benzyl-2-n-propyl-1H-purine-6-yl, 1-benzyl-2-n-butyl-1H-purine-6-yl, 1-benzyl-2-n-pentyl-1H-purine-6-yl, 1-benzyl-2-n-hexyl-1H-purine-6-yl, 2-n-butyl-1-(1-phenylethyl)-1H-purine-6-yl, 2-n-butyl-1-(2-phenylethyl)-1H-purine-6-yl, 2-n-butyl-1-(3-phenylpropyl)-1H-purine-6-yl, 2-n-butyl-1-(4-phenylbutyl)-1H-purine-6-yl, 2-n-butyl-1-(5-phenyl pentyl)-1H-purine-6-yl, 2-n-butyl-1-(6-phenylhexyl)-1H-purine-6-yl, 1,2-dimethyl-1H-purine-6-yl, 2-ethyl-1-methyl-1H-purine-6-yl, 1-

methyl-2-n-propyl-1H-purine-6-yl, 2-n-butyl-1-methyl-1H-purine-6-yl, 1-methyl-2-n-pentyl-1H-purine-6-yl, 2-n-hexyl-1-methyl-1H-purine-6-yl and the like.

(0033)

The amide derivatives represented by aforesaid general formula (1) are useful as NO synthase inhibitor, in particular the agent which inhibits iNOS selectively, for example for prevention and therapy of septicaemia, endotoxin shock, chronic rheumatoid arthritis and the like, more preferably, as septicemia prevention and treatment agent and endotoxin shock improving agent. In particular NO synthase inhibitor of this invention has an advantage that it is almost free from the side effect which is seen in the prior art NO synthase inhibitor.

(0034)

As amide derivative including by general formula (1) and which is preferred as NO synthase inhibitor effective ingredient, the compound wherein A is benzene ring and R5 is hydrogen atom in the said general formula (1) can be exemplified.

(0035)

Among these, the one wherein heterocyclic group represented by R4 is thieno[3,2-d]pyrimidin-4-yl group containing lower alkyl group at 2-position as substituent, pyrazolo[1,5-a]-1,3,5-triazin-4-yl group containing lower alkyl group at 2-position as substituent or 9H-purin-6-yl group contains lower alkyl group at 2-position is ideal, even among them, the one wherein R1, R2 and R3 are the same or different and denote hydrogen atom, lower alkoxy group or halogen atom is more preferred.

(0036)

For example N-(2-n-butyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl)-2,3,4-trimethoxy benzamide, N-(2-n-butyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl)-2-methoxy benzamide, N-(2-n-butyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl)-2,4-dichlorobenzamide, N-(2-n-butyl-9H-purine-6-yl)-3,4,5-trimethoxy benzamide, N-(2-n-butyl thieno[3,2-d]pyrimidine-4-yl)-3,4,5-trimethoxy benzamide, N-(2-n-butyl thieno[3,2-d]pyrimidine-4-yl)-2-chlorobenzamide, N-(2-n-butyl thieno[3,2-d]pyrimidine-4-yl)-2,4-dichlorobenzamide and N-(2-n-butyl thieno[3,2-d]pyrimidine-4-yl)-3-methoxy benzamide can be exemplified as embodiment example of particular preferred compound as NOS inhibitor effective ingredient of this invention. Among these, N-(2-n-butyl thieno[3,2-d] pyrimidine-4-yl)-3,4,5-trimethoxy benzamide is most preferred.

(0037)

Effective ingredient compound represented by general formula (1) of this invention is possible to produce using various process. As example thereof, for example the process in accordance with

aforesaid WO bulletin may be proposed. In further embodiment, the process to react corresponding heterocyclic amine derivative and acid halide can be exemplified. Embodiment example thereof is explained in detail in the section of Examples described hereinafter.

(0038)

As embodiment of effective ingredient compound of NOS inhibitor of this invention obtained in this way, each compound shown in the section of later-described Examples (it is denoted as Examples 1-120) can be exemplified.

(0039)

Effective ingredient compound represented by general formula (1) of this invention can be made into the pharmacologically acceptable acid addition salts, and such salts are also included in effective ingredient compound of this invention. As the acid which can form the aforesaid acid addition salts, for example inorganic acid such as hydrochloric acid, hydrobromic acid, sulfuric acid or the like, organic acid such as oxalic acid, fumaric acid, maleic acid, tartaric acid, citric acid or the like can be exemplified, and forming reaction of this acid addition salt can be carried out by a normal method.

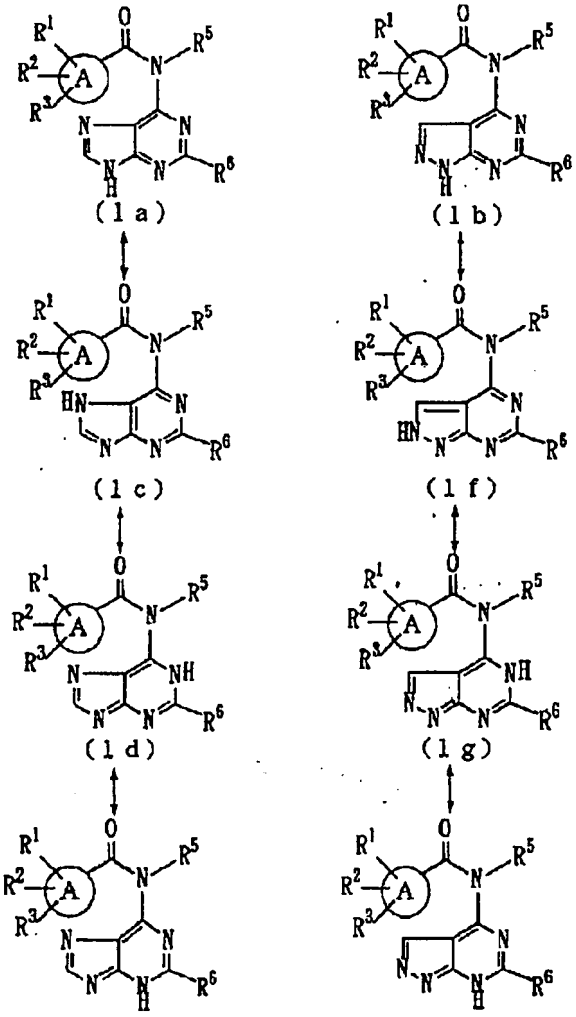
(0040)

Moreover, among effective ingredient compounds of this invention, the compound wherein R5 is hydrogen atom can be made into alkali metal salt such as sodium salt, potassium salt and the like, alkaline earth metal salt such as calcium salt, magnesium salt and the like or else a cuprate or the like according to normal method, and such salts are also included in the effective ingredient of this invention.

(0041)

Moreover, among effective ingredient compounds of this invention, the following compound (1a) and compound (1b) are thought to have the following resonance structures (1c)-(1e) and (1f)-(1h) respectivelly, and it can be represented by any structural formula.

(0042)



(0043)

NOS inhibitor of this invention is made into a general drug preparation composition using at least one species selected from the compound represented by general formula (1) and the salts thereof as effective ingredient together with the suitable preparation carrier, and used practically.

(0044)

As the aforesaid preparation carrier, corresponding to conditions of use of preparation, usually used diluent or excipient such as lubricant, filler, expander, binding agent, humectant, disintegrating agent, surface active agent, can be given as example and these are suitably selected and used corresponding to administration unit form of preparation to be obtained.

(0045)

As administration unit form of the aforesaid drug preparation, various forms can be selected corresponding to therapy objective, and, as representative examples thereof, tablet, pill, powder, liquid agent, suspension, emulsion, granule, encapsulated formulation, suppository, injection (liquid agent, suspension or the like), ointment and the like may be proposed.

(0046)

When forming into tablet, as the aforesaid preparation carrier, for example excipient such as lactose, refined sugar, sodium chloride, glucose, urea, starch, calcium carbonate, kaolin, crystalline cellulose, silica, potassium phosphate and the like; binding agent such as water, ethanol, propanol, single syrup, glucose liquid, starch liquid, gelatin solution, carboxymethylcellulose, hydroxypropylcellulose, methyl cellulose, polyvinylpyrrolidone and the like; disintegrating agent such as carboxymethylcellulose sodium, carboxymethylcellulose calcium, low degree of substitution hydroxypropylcellulose, dry starch, sodium alginate, agar powder, laminaran powder, sodium bicarbonate, calcium carbonate and the like; surfactant such as polyoxyethylene sorbitan fatty acid esters, sodium lauryl sulfate, stearic acid monoglyceride and the like; disintegration inhibitor such as refined sugar, stearin, cacao butter, hydrogenated oil or the like; adsorption enhancer such as quaternary ammonium salt group, sodium lauryl sulfate and the like; moisture retaining agent such as glycerol, starch and the like; adsorbent such as starch, lactose, kaolin, bentonite, colloidal silica or the like; lubricant such as purified talc, stearate, boric acid powder, polyethyleneglycol and the like can be used.

(0047)

Further the tablet can be made into the tablet coated with ordinary agent coating in accordance with requirements, for example sugar coated tablet, gelatin encapsulation tablet, enteric coated tablet, film coating tablet or double tablet, multilayer tablet.

(0048)

When formed into the form of a pill, excipient such as for example carrier such as glucose, lactose, starch, cacao butter, hardened vegetable oil, kaolin, talc and the like; binding agent such as powdered gum arabic, tragacanth powder, gelatin, ethanol and the like; disintegrating agent such as laminaran, agar and the like can be used as preparation carrier.

(0049)

When formed into a form of suppository, as preparation carrier, for example polyethyleneglycol,

cacao butter, higher alcohol, esters of higher alcohol, gelatin, semi-synthetic glyceride and the like can be used.

(0050)

Encapsulated formulation is usually prepared according to normal method, by mixing effective ingredient compound of this invention with the various preparation carrier exemplified above and packing into hard gelatin capsule, soft capsule and the like.

(0051)

When prepared as injection agent such as liquid agent, emulsion, suspension and so on, such materials are sterilized and preferably made isotonic with blood, and when formed into such forms, as a diluent, for example, water, ethanol, macrogol, propylene glycol, ethoxylation isostearyl alcohol, polyoxyisosteary alcohol, polyoxyethylene sorbitan fatty acid ester species as can be used. Moreover, in this case, sufficient sodium chloride, dextrose or glycerol to form an isotonic solution may be contained in agent of this invention, and moreover ordinary solubilizer, buffer agent, analgesic or the like may be added.

(0052)

Furthermore, in agent of this invention, colorant, preservative, odorant, flavor agent, sweetener and so on and other pharmaceutical can be contained in accordance with requirements.

(0053)

When prepared into a form of ointment such as paste, cream, gel and the like, for example white petrolatum, paraffin, glycerol, cellulose derivative, polyethyleneglycol, silicone, bentonite and the like can be used as diluent.

(0054)

The amount of effective ingredient compound to be contained in the agent of this invention is suitably selected from a wide range without restriction in particular, but usually one containing an amount of about 1-70 wt.% approximately in the drug preparation is satisfactory.

(0055)

Administration method of the aforesaid drug preparation is not limited in particular, and it is determined corresponding to various formulations, age of patient, the distinction of sex, other conditions, degree of disease or the like. For example, tablet, pill, liquid agent, suspension, emulsion, granule and encapsulated formulation are administered orally, and injection is used

alone or mixed with ordinary adjuvant fluid such as dextrose, amino acid or the like, and administered intravenously, and further in accordance with requirements, it may be administered alone intramuscularly, intracutaneously, subcutaneously or intraperitoneally, and, the suppository is administered rectally.

(0056)

The dose of the aforesaid drug preparation is suitably selected in accordance with the method of use thereof, age of patient, the distinction of sex, other conditions, degree of disease or the like, but usually an amount of the effective ingredient compounds of about 0.5-20 mg per 1 kg bodyweight per day is satisfactory, and said preparation can be administered by being divided 1-4 times per day.

(0057)

Examples

Hereinafter, in order to further describe in detail this invention, production examples of raw material compound for production of compound comprising an effective ingredient in NOS inhibitor of this invention are nominated as Reference Examples, then production examples of the said effective ingredient compound are nominated as Examples, moreover preparation examples of NOS inhibitor of this invention are nominated as Preparation Examples, and Pharmacological Test Examples are nominated at the end.

(0058)

Reference Example 1

Production of 4-amino-2-n-butyl thieno[3,2-d]pyrimidine

To anhydrous pyridine 50 ml solution of 3-aminothiophene-2-carboxylic acid methyl ester 5.0 g was added n-pentanoic acid chloride 3.8 ml at 0°C, and the mixture was stirred at 0°C for one hour, and thereafter, the mixture was stirred at room temperature for two hours. The reaction solution was concentrated, diluted with ethyl acetate, and washed successively with 1N hydrochloric acid, saturated aqueous sodium bicarbonate and saturated aqueous sodium chloride solution. Ethyl acetate was distilled under reduced pressure, and the residue was purified by silica gel column chromatography (eluted with n-hexane : ethyl acetate = 5 : 1) and colourless oily substance 7.0 g of 3-pentanoyl aminothiophene-2-carboxylic acid methyl ester was obtained.

(0059)

To dimethoxyethane 5 ml solution of compound 4.0 g obtained as above was added 25 % ammonia water solution 20 ml and heated at 100°C in a sealed tube for 24 hours. The reaction solution was concentrated under reduced pressure and extraction was carried out with

dichloromethane. The organic layer was recovered and concentrated under reduced pressure, and recrystallisation was carried out from dichloromethane-n-hexane, and colourless crystals 1.35 g of 5-n-butyl-7-hydroxy thieno[3,2-d]pyrimidine was obtained.

(0060)

In the obtained crystals 1.35 g dissolved in toluene 1 ml were added phosphorus oxychloride 2.4 ml and triethylamine 1.3 ml, and the mixture was stirred at 115°C for 12 hours. The reaction liquor was concentrated under reduced pressure, and it was poured into iced water, and it was filtered with celite after neutralization with sodium acetate. The filtrate was extracted with ethyl acetate, and the organic layer was recovered and washed successively with water and saturated aqueous sodium chloride solution, and concentration was carried out under reduced pressure. The residue was purified by silica gel column chromatography (eluted with n-hexane : ethyl acetate = 4 : 1) and colourless oily substance 1.4 g of 5-n-butyl-7-chloro thieno[3,2-d]pyrimidine was obtained.

(0061)

To dimethoxyethane 3 ml solution of the obtained oily substance 1.4 g was added 25 % ammonia water solution 15 ml and heated at 80°C in a sealed tube for 20 hours. On completion of the reaction, it was cooled with water, and the precipitated crystals were recovered by filtration, washed with water and then dried, and colourless crystals 1.2 g of the target compound were obtained.

(0062)

Moreover, in the same way as described above, 4-amino-2-n-propyl thieno[3,2-d]pyrimidine was produced.

(0063)

Reference Example 2

Production of 4-amino-6-n-butyl-1H-pyrazolo[3,4-d]pyrimidine

To anhydrous DMF 50 ml solution of 3-amino-4-cyanopyrazole 5 g, ortho n-pentanoic acid trimethyl 12 ml was added, and the mixture was stirred at 90°C for 20 minutes. The reaction solution was diluted with ethyl acetate, washed successively with water and saturated aqueous sodium chloride solution, and thereafter it was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (eluted with n-hexane : ethyl acetate = 2 : 1) and colourless oily substance 6.5 g of 4-cyano-3-(N-[1-methoxy pentylidene] amino) pyrazole was obtained

(0064)

Methanol solution (about 7 %) 50 ml of ammonia was added to compound 6.5 g obtained as above, and the mixture was stirred at room temperature for 36 hours. On completion of the reaction, it was concentrated under reduced pressure, and the residue was recrystallised from ethanol-n-hexane, and colourless crystals 4.1 g of the target compound were obtained.

(0065)

Reference Example 3

Production of 4-amino-2-benzyl oxycarbonyl-6-n-butyl-2H-pyrazolo[3,4-d]pyrimidine

To anhydrous DMF 7.5 ml solution of compound 750 mg obtained in Reference Example 2, triethylamine 1.1 ml and benzyl oxycarbonyl chloride (about 30 % toluene solution) 3.4 ml were respectively added at 0°C, and the mixture was stirred at 0°C for one hour. The reaction liquor was introduced into iced water, and the precipitated crystals were recovered by filtration, washed with diethyl ether, and purified by silica gel column chromatography (eluted with chloroform : methanol = 40 : 1 to 10 : 1), thereafter recrystallization was further carried out from ethanol-n-hexane, and colourless crystals 3.0 mg of the target compound were obtained.

(0066)

Reference Examples 4 and 5

Production of 4-amino-2-benzyl-6-n-butyl-2H-pyrazolo[3,4-d]pyrimidine and 4-amino-1-benzyl-6-n-butyl-1H-pyrazolo[3,4-d]pyrimidine

The crude product obtained by the treatment in the same way as in Reference Example 3 using the compound obtained in Reference Example 2, benzyl bromide and sodium hydride as base was recrystallised from dichloromethane-diethyl ether, and colourless crystals of 4-amino-2-benzyl-6-n-butyl-2H-pyrazolo[3,4-d]pyrimidine were obtained.

(0067)

On the other hand, recrystallization mother liquor was concentrated, the residue was purified by column chromatography (eluted with n-hexane : ethyl acetate = 2 : 3), and thereafter it was further recrystallised from diethyl ether-n-hexane, and colourless crystals of 4-amino-1-benzyl-6-n-butyl-1H-pyrazolo[3,4-d]pyrimidine were obtained.

(0068)

Reference Example 6

Production of 6-amino-2-n-butyl-9H-purine

To anhydrous DMF 24 ml solution of 4-amino-5-cyanoimidazole 10 g, ortho-n-pentanoic acid trimethyl 24 ml was added, and the mixture was stirred at 90°C for 20 minutes. The reaction solution was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (eluted with ethyl acetate) and it was further recrystallised from ethyl

acetate-n-hexane, and colourless crystals 17.7 g of 5-cyano-4-(N-[1-methoxy pentylidene] amino) imidazole was obtained.

(0069)

Methanol solution (2N) 100 ml of ammonia was added to compound 15 g obtained as above, and the mixture was stirred at room temperature for six days. On completion of the reaction, the precipitated crystals were recovered by filtration, washed with methanol, and thereafter dried, and colourless crystals 9.5 g of the target compound were obtained. Moreover, the filtrate was concentrated, and the residue was recrystallised from ethanol-n-hexane, and colourless crystals 3.0 g of the target compound were further obtained.

(0070)

Reference Example 7

Production of 6-amino-9-benzyl oxycarbonyl-2-n-butyl-9H-purine

To anhydrous DMF 100 ml solution of compound 10 g obtained in Reference Example 6, triethylamine 22 ml and benzyl oxycarbonyl chloride (about 30 % toluene solution) 45 ml were respectively added at 0°C, and the mixture was stirred at 0°C for five hours. The reaction liquor was discharged into iced water, extracted with chloroform, and the chloroform layer was washed with saturated aqueous sodium chloride solution, and concentration was carried out under reduced pressure. The residue was recrystallised from chloroform-diethyl ether, and colourless crystals 8.5 g of the target compound were obtained. Moreover, the mother liquor was concentrated, and the residue was purified by silica gel column chromatography (eluted with chloroform : methanol = 20 : 1) and thereafter, it was recrystallised from chloroform-diethyl ether, and colourless crystals 2.4 g of the target compound were further obtained.

(0071)

Reference Example 8

Production of 6-amino-1-benzyl-2-n-butyl-1H-purine

5-cyano-4-(N-[1-methoxy pentylidene] amino) imidazole 5 g produced in Reference Example 6 was dissolved in methanol 50 ml, and benzylamine 3.2 ml was added, and the mixture was stirred at 50°C for two hours. The mixture was allowed to cool, and thereafter the precipitated crystals were recovered by filtration and washed with diethyl ether, and colourless crystals 6.2 g of the target compound were obtained.

(0072)

Reference Example 9

Production of 6-amino-2-n-butyl-1-methyl-1H-purine

In the same way as in Reference Example 8, colourless crystals of the target compound were obtained.

(0073)

Reference Example 10

Production of 6-amino-7-benzyl-2-n-butyl-7H-purine

Using 1-benzyl-4-amino-5-cyanoimidazole, colourless crystals of the target compound were obtained in the same way as in Reference Example 6.

(0074)

Reference Example 11

Production of 6-amino-9-benzyl-2-n-butyl-9H-purine

Using 3-benzyl-4-amino-5-cyanoimidazole, colourless crystals of the target compound were obtained in the same way as in Reference Example 6.

(0075)

Reference Example 12

Production of 4-amino-2-n-butyl pyrazolo[1,5-a]-1,3,5-triazine

To anhydrous DMF 250 ml solution of 3-aminopyrazole 50 g, ortho-n-pentanoic acid trimethyl ester 120 ml was added and the mixture was stirred at 70°C for 22 hours. On completion of the reaction, it was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (eluted with dichloromethane : methanol = 50 : 1 to 20 : 1) and colourless oily substance 60 g of 3-(N-[1-methoxy pentylidene] amino) pyrazole was obtained

(0076)

The compound 60 g obtained as above was dissolved in methanol 300 ml, and cyanamide 15.3 g was added, and the mixture was stirred at 60°C for 17 hours. On completion of the reaction, it was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (eluted with dichloromethane : methanol = 50 : 1) and it was further recrystallised from diisopropyl ether, and colourless crystals 36.4 g of the target compound were obtained. Moreover, recrystallization mother liquor was purified in the same way, and colourless crystals 7 g of the target compound were obtained.

(0077)

Reference Example 13

Production of 4-amino-2-phenylpyrazoro[1,5-a]-1,3,5-triazine

In the same way as in Reference Example 12, colourless crystals of the target compound were obtained.

(0078)

Reference Examples 14-20

Furthermore, in the same way as in Reference Example 12, crystals of the following respective compound were obtained.

(0079)

Reference Example 14

4-amino-2-methylpyrazolo[1,5-a]-1,3,5-triazine.

Reference Example 15

4-amino-2-ethyl pyrazolo[1,5-a]-1,3,5-triazine.

Reference Example 16

4-amino-2-n-propyl pyrazolo[1,5-a]-1,3,5-triazine.

Reference Example 17

4-amino-2-n-pentyl pyrazolo[1,5-a]-1,3,5-triazine.

Reference Example 18

4-amino-2-benzyl pyrazolo[1,5-a]-1,3,5-triazine.

Reference Example 19

4-amino-2-n-butyl-8-phenylpyrazoro[1,5-a]-1,3,5-triazine.

Reference Example 20

4-amino-2-n-butyl-8-(4-phenylthio phenyl) pyrazolo[1,5-a]-1,3,5-triazine.

(0080)

Example 1

Production of N-(2-n-butyl thieno[3,2-d]pyrimidine-4-yl)-3,4,5-trimethoxy benzamide

3,4,5-trimethoxy benzoyl chloride 270 mg was added to anhydrous pyridine 4 ml solution of compound 200 mg obtained in Reference Example 1 at 0°C, and thereafter, the mixture was stirred at 0°C for one hour and then at room temperature for three days. The reaction liquor was diluted with chloroform, and it was washed successively with 10 % hydrochloric acid, saturated aqueous sodium bicarbonate and saturated aqueous sodium chloride solution, and thereafter concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluted with n-hexane : ethyl acetate = 3 : 2) and it was further recrystallised

from n-hexane, and colourless crystals 1.0 mg of the target compound were obtained. The structure and mp. of the obtained compound are shown in Table 1.

(0081)

Examples 2-8

In the same way as in Example 1, each compound in accordance with Table 1 was produced. The structure and mp. of the obtained compound are shown in Table 1. Moreover, for oily substance, ^{1}H-NMR spectral data (δ: ppm value; solvent = deuterated chloroform; internal standard = tetramethylsilane) is shown.

(0082)

Example 9

Production of N-(2-n-butyl-9H-purine-6-yl)-3,4,5-trimethoxy benzamide

3,4,5-trimethoxy benzoyl chloride 5.3 g was added to anhydrous pyridine 50 ml solution of compound 5 g obtained in Reference Example 7 at 0°C, and thereafter, the mixture was stirred at 0°C for two hours and then at room temperature for five days. The reaction liquor was diluted with chloroform and was washed with 10 % hydrochloric acid, and thereafter, extraction was carried out with 5 % sodium hydroxide aqueous solution. The aqueous layer was recovered, and was neutralized with 10 % hydrochloric acid and extraction was carried out with chloroform. The chloroform layer was recovered, washed successively with water and saturated aqueous sodium chloride solution, and concentration was carried out under reduced pressure. The residue was purified by silica gel column chromatography (eluted with chloroform : methanol = 50 : 1 to 20 : 1) and it was further recrystallised from dichloromethane-diethyl ether, and colourless crystals 2.7 g of the target compound were obtained. The structure and mp. of the obtained compound are shown in Table 1.

(0083)

Example 10

Production of N-(6-n-butyl-1H-pyrazolo[3,4-d]pyrimidine-4-yl)-3,4,5-trimethoxy benzamide

3,4,5-trimethoxy benzoyl chloride 106 mg was added to anhydrous pyridine 2 ml solution of compound 100 mg obtained in Reference Example 3 at 0°C, and thereafter, the mixture was stirred at 0°C for one hour and then at room temperature for one hour. The reaction liquor was diluted with ethyl acetate, washed successively with 10 % hydrochloric acid, saturated aqueous sodium bicarbonate and saturated aqueous sodium chloride solution, and concentration was carried out under reduced pressure. The residue was purified by silica gel column chromatography (eluted with chloroform : ethyl acetate = 1 : 1) and it was further recrystallised from dichloromethane-n-hexane, and colourless crystals 150 mg was obtained.

(0084)

The obtained crystals were dissolved in ethanol 10 ml, and 10 % palladium-carbon 20 mg was added, and it was stirred overnight at room temperature in hydrogen gas. Palladium-carbon was eliminated by filtration with celite, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluted with chloroform : methanol = 50 : 1) and it was further recrystallised from ethyl acetate-n-hexane, and colourless crystals 60 mg of the target compound were obtained. The structure and mp. of the obtained compound are shown in Table 1.

(0085)

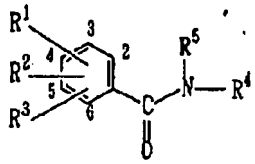
Examples 11 and 12

Production of N-(9-benzyl-2-n-butyl-9H-purine-6-yl)-3,4,5-trimethoxy benzamide and N-(9-benzyl-2-n-butyl-9H-purine-6-yl)-N-(3,4,5-trimethoxy benzoyl)-3,4,5-trimethoxy benzamide

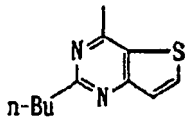
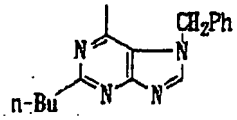
To anhydrous pyridine 30 ml solution of compound 1.5 g obtained in Reference Example 11, 3,4,5-trimethoxy benzoyl chloride 1.85 g was added at room temperature and stirred at room temperature for six days. The reaction liquor was diluted with dichloromethane, washed successively with 10 % hydrochloric acid, saturated aqueous sodium bicarbonate, water and saturated aqueous sodium chloride solution, and concentration was carried out under reduced pressure. The residue was purified by silica gel column chromatography (eluted with dichloromethane : methanol = 100 : 1 to 50 : 1) and the earlier fraction was recrystallised from n-hexane, and colourless crystals 0.75 g of N-(9-benzyl-2-n-butyl-9H-purine-6-yl)-N-(3,4,5-trimethoxy benzoyl)-3,4,5-trimethoxy benzamide was obtained. Further the later fraction was recrystallised from n-hexane, and colourless crystals 0.72 g of N-(9-benzyl-2-n-butyl-9H-purine-6-yl)-3,4,5-trimethoxy benzamide was obtained. The structure and mp. of the obtained compound are shown in Table 1.

(0086)

Table 1

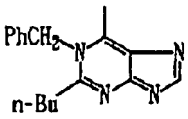
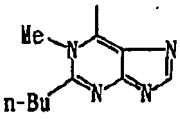
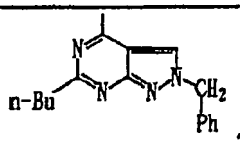
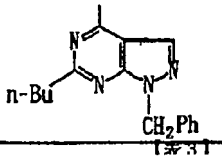


Me = methyl group, n-Bu = n-butyl group, Ph = phenyl group

Example	R^1	R^2	R^3	R^4	R^5	mp (°C)
1	3-OMe	4-OMe	5-OMe		H	95－97
2	3-OMe	4-OMe	5-OMe		H	185－187

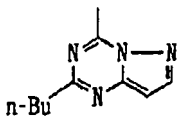
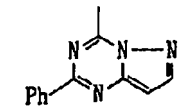
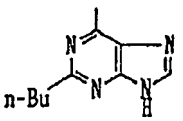
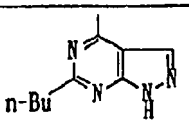
(0087)

Table 1 (continued)

Example	R^1	R^2	R^3	R^4	R^5	mp (°C)
3	3-OMe	4-OMe	5-OMe		H	Oily substance ^{1}H-NMR
4	3-OMe	4-OMe	5-OMe		H	159－161
5	3-OMe	4-OMe	5-OMe		H	217－219 hydrochloride
6	3-OMe	4-OMe	5-OMe		H	46－48

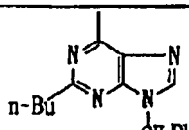
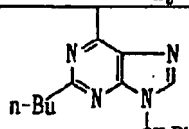
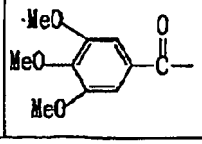
(0088)

Table 1 (continued)

Example	R 1	R 2	R 3	R 4	R 5	mp (°C)
7	3-OMe	4-OMe	5-OMe		H	124－126
8	3-OMe	4-OMe	5-OMe		H	174－176
9	3-OMe	4-OMe	5-OMe		H	132－134
10	3-OMe	4-OMe	5-OMe		H	151－153

(0089)

Table 1 (continued)

Example	R 1	R 2	R 3	R 4	R 5	mp (°C)
11	3-OMe	4-OMe	5-OMe		H	92－94
12	3-OMe	4-OMe	5-OMe			112－114

(0090)

Moreover, ^{1}H-NMR analysis value of compound of Example 3 in accordance with the aforesaid Table 1 is as follows.

(0091)

0.90 (3H, t, J = 7.2), 1.3-1.5 (2H, m), 1.8-1.9 (2H, m), 2.87 (2H, t, J = 7.7), 3.62 (6H, s), 3.86 (3H, s), 5.6-6.2 (2H, brs), 7.18 (2H, d, J = 6.9), 7.2-7.4 (5H, m), 8.13 (1H, s), 12.3-12.5 (1H, brs).

(0092)

Examples 13-53

Furthermore, in the same way as in Example 1, each compound in accordance with Table 2 was produced. The structure and mp. of the obtained compound are shown in Table 2. Moreover, about an oily substance, ^{1}H-NMR spectral data (δ: ppm value; solvent=DMSO-d6; internal standard=tetramethylsilane) is denoted.

(0093)

Example 54

Production of N-(2-n-butyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl)-2-methylsulfinyl benzamide

To acetic acid 18 ml solution of compound. 1.2 g obtained in Example 47, 30 % hydrogen peroxide water 0.44 ml was added, and the mixture was stirred at room temperature for four hours. On completion of the reaction, 10 % sodium hydroxide aqueous solution was added to be neutralized, and extraction was carried out with dichloromethane. The organic layer was recovered, washed successively with water and saturated aqueous sodium chloride solution, and concentration was carried out under reduced pressure. Diethyl ether was added to the residue, and the precipitated crystals were recovered by filtration, and it was recrystallised from dichloromethane-diethyl ether, and colourless crystals 0.48 g of the target compound were obtained. The structure and mp. of the obtained compound are shown in Table 2.

(0094)

Example 55

Production of N-(2-n-butyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl)-2-methylsulfonyl benzamide

Into the compound 1.0 g obtained in Example 54 dissolved in chloroform 1 ml was added dropwise metachloro perbenzoic acid 1.44 g of chloroform 15 ml solution at -78°C and the mixture was stirred at the same temperature for 45 minutes, and then it was further stirred at 0°C for one hour. The reaction liquor was diluted with dichloromethane, washed successively with aqueous sodium bicarbonate and saturated aqueous sodium chloride solution, and concentration was carried out under reduced pressure. The residue was purified by silica gel column chromatography (eluate; chloroform to chloroform : methanol = 40 : 1) and it was further recrystallised from ethyl acetate-n-hexane, and colourless crystals 0.95 g of the target compound were obtained. The structure and mp. of the obtained compound are shown in Table 2.

(0095)

Example 56

Production of N-(2-n-butyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl)-2-hydroxybenzamide

10 % sodium hydroxide aqueous solution 2.0 ml was added at 0°C to ethanol 15 ml suspension of compound 1.5 g obtained in Example 44, and the mixture was stirred at the same temperature for one hour. 10 % hydrochloric acid 2.2 ml and water 80 ml were added to the reaction liquor,

and the precipitated crystals were recovered by filtration, washed with water, and thereafter recrystallised from 60 % water-containing ethanol, and colourless crystals 1.12 g of the target compound were obtained. The structure and mp. of the obtained compound are shown in Table 2.

(0096)

Example 57

Production of N-(2-n-butyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl)-4-hydroxybenzamide

In the same way as in Example 56, target compound was produced from the compound obtained in Example 46. The structure and mp. of the obtained compound are shown in Table 2.

(0097)

Example 58

Production of N-(8-bromo-2-n-butyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl)-3,4,5-trimethoxy benzamide

The compound 1.0 g obtained in Example 7 was dissolved in 1,2-dimethoxyethane-water (3 : 1) 20 ml, and thereto was added NBS 0.51 g at 0°C, and the mixture was stirred at 0°C for one hour. The liquid reaction mixture was diluted with water, and the precipitated crystals were recovered by filtration. The obtained crude crystals were washed with water, recrystallised from methanol-water, and colourless crystals 0.94 g of the target compound were obtained. The structure and mp. of the obtained compound are shown in Table 2.

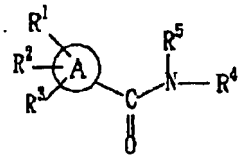
(0098)

Examples 59-62

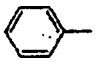
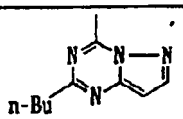
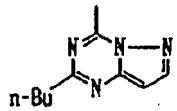
Each compound shown as Examples 59-62 in Table 2 was respectively isolated from the earlier fraction of silica gel chromatography in Examples 7, 13, 14 and 25. The structure and mp. of the obtained compound are shown in Table 2.

(0099)

Table 2

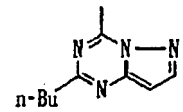
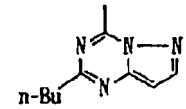
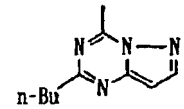
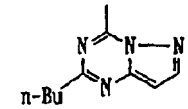


Me = methyl group, Et = ethyl group, n-Pr = n-propyl group, n-Bu = n-butyl group, t-bu = t-butyl group, n-Pe = n-pentyl group, Ac = acetyl group, Ph = phenyl group.

Example	Ⓐ—	R^1	R^2	R^3	R^4	R^5	mp (°C)
1-3		2-CF_3	H	H		H	112 or more (decomp.) Na salt
1 4		2-Cl	H	H		H	63 or more (decomp.) Na salt

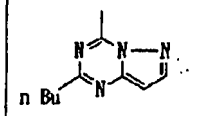
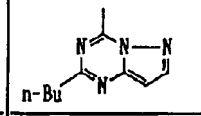
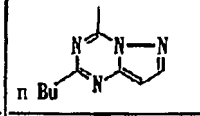
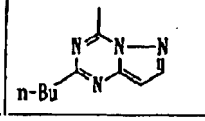
(0100)

Table 2 (continued)

Example	Ⓐ—	R^1	R^2	R^3	R^4	R^5	mp (°C)
1 5		2-Cl	4-Cl	H		H	1 0 0 – 1 0 2
1 6		2-OMe	H	H		H	1 1 9 – 1 2 1
1 7		3-Cl	H	H		H	1 1 6 – 1 1 8
1 8		4-Cl	H	H		H	7 4 – 7 6

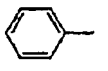
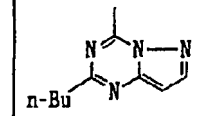
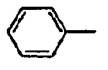
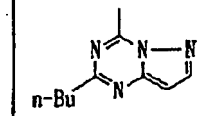
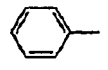
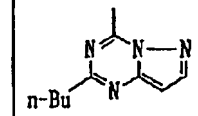
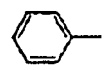
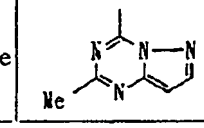
(0101)

Table 2 (continued)

Example	Ⓐ—	R^1	R^2	R^3	R^4	R^5	mp.°C
19		3-F	H	H		H	96－98
20		H	H	H		H	82－84
21		3-OMe	H	H		H	75－77
22		4-OMe	H	H		H	91－93

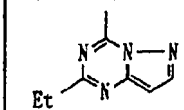
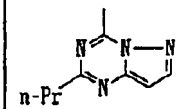
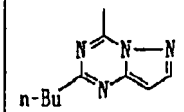
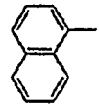
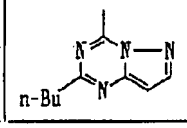
(0102)

Table 2 (continued)

Example	Ⓐ—	R^1	R^2	R^3	R^4	R^5	mp (°C)
23		2-Cl	5-Cl	H		H	134 or more (decomp.) hydrochloride
24		2-Br	H	H		H	135 or more (decomp.) hydrochloride
25		2-NO_2	H	H		H	89－91
26		3-OMe	4-OMe	5-OMe		H	165－167

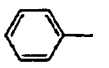
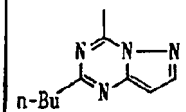
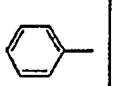
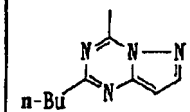
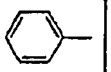
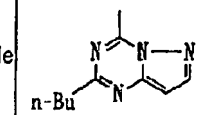
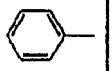
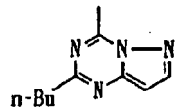
(0103)

Table 2 (continued)

Example	A	R^1	R^2	R^3	R^4	R^5	mp (°C)
27		3-OMe	4-OMe	5-OMe		H	148-150
28		3-OMe	4-OMe	5-OMe		H	145-147
29		4-t-Bu	H	H		H	96-98
30		H	H	H		H	100-102

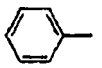
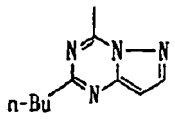
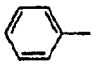
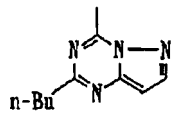
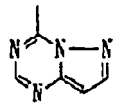
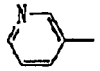
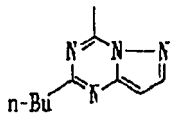
(0104)

Table 2 (continued)

Example	A	R^1	R^2	R^3	R^4	R^5	mp (°C)
31		4-CF_3	H	H		H	90-92
32		2-OMe	4-OMe	H		H	136-138
33		2-OMe	3-OMe	4-OMe		H	142-144
34		4-Ph	H	H		H	119-121

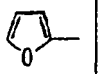
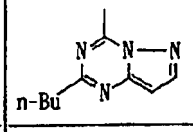
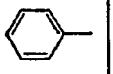
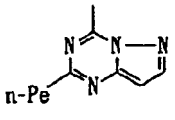
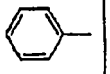
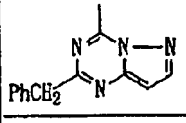
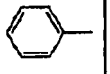
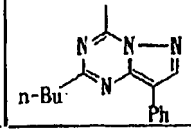
(0105)

Table 2 (continued)

Example	A	R^1	R^2	R^3	R^4	R^5	mp (°C)
35		2-OPh	H	H		H	147-149
36		4-O-n-Bu	H	H		H	116-118
37		3-OMe	4-OMe	5-OMe		H	213-215
38		H	H	H		H	76-78

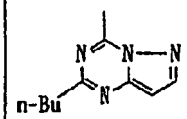
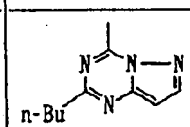
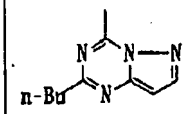
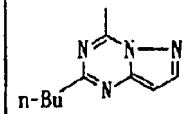
(0106)

Table 2 (continued)

Example	A	R^1	R^2	R^3	R^4	R^5	mp (°C)
39		H	H	H		H	100-102
40		3-OMe	4-OMe	5-OMe		H	115-117
41		3-OMe	4-OMe	5-OMe		H	164-166
42		3-OMe	4-OMe	5-OMe		H	150-152

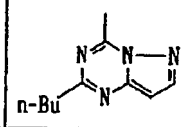
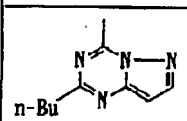
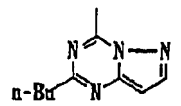
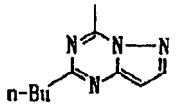
(0107)

Table 2 (continued)

Example	A	R^1	R^2	R^3	R^4	R^5	mp (°C)
43		2-Me	H	H		H	Oily substance ^{1}H-NMR
44		2-OAc	H	H		H	123-125
45		3-Cl	4-Cl	H		H	113-115
46		4-OAc	H	H		H	140-142

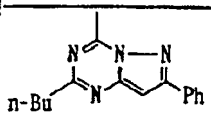
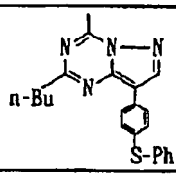
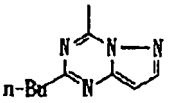
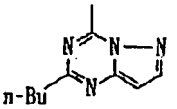
(0108)

Table 2 (continued)

Example	A	R^1	R^2	R^3	R^4	R^5	mp (°C)
47		2-SMe	H	H		H	111-113
48		2-OEt	H	H		H	153-155
49		3-OMe	4-OMe	H		H	113-115
50		2-OMe	3-OMe	H		H	147-149

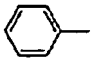
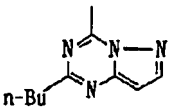
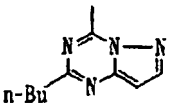
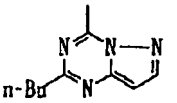
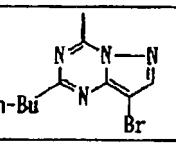
(0109)

Table 2 (continued)

Exaxmple	A	R^1	R^2	R^3	R^4	R^5	mp (°C)
51		3-OMe	4-OMe	5-OMe		H	172-174
52		3-OMe	4-OMe	5-OMe		H	150-152
53		3-Me	H	H		H	76-78
54		2-SOMe	H	H		H	168-170

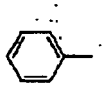
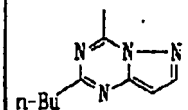
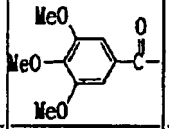
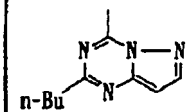
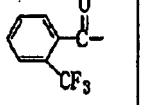
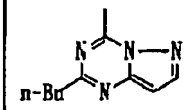
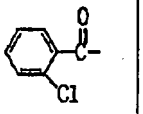
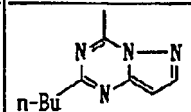
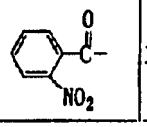
(0110)

Table 2 (continued)

Example	A	R^1	R^2	R^3	R^4	R^5	mp (°C)
55		2-SO_2Me	H	H		H	105-107
56		2-OH	H	H		H	125-127
57		4-OH	H	H		H	169-171
58		3-OMe	4-OMe	5-OMe		H	160-162

(0111)

Table 2 (continued)

Example	Ⓐ	R^1	R^2	R^3	R^4	R^5	mp (°C)
59		3-OMe	4-OMe	5-OMe			93-95
60		2-CF_3	H	H			128-130
61		2-Cl	H	H			90-92
62		2-NO_2	H	H			146-148

(0112)

Moreover, ^{1}H-NMR analysis value of compound of Example 43 in accordance with the aforesaid Table 2 is as follows.

(0113)

0.88 (3H, t, J = 7.4), 1.2-1.4 (2H, m), 1.5-1.7 (2H, m), 2.47 (3H, s), 2.66 (2H, t, J = 6.9), 6.55 (1H, d, J = 2.0), 7.2-7.4 (2H, m), 7.43 (1H, t, J = 7.4), 7.62 (1H, d, J = 6.9), 8.21 (1H, d, J = 2.0), 11.6-11.9 (1H, brs).

(0114)

Examples 63-75

Furthermore, in the same way as in Example 1, each compound in accordance with Table 3 was produced. The structure and mp. of the obtained compound are shown in Table 3.

(0115)

Examples 76-82

Each compound shown as Examples 76-82 in Table 3 was respectively isolated from the earlier fraction of silica gel chromatography in Examples 63-64 and Examples 68-72. The structure and mp. of the obtained compound are shown in Table 3.

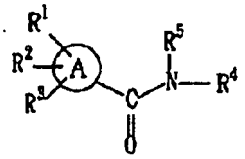
(0116)

Example 83

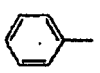
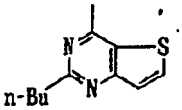
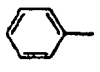
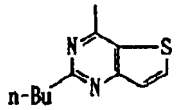
From compound obtained in Example 75, compound in accordance with Table 3 was produced in the same way as in Example 56. The structure and mp. of the obtained compound are shown in Table 3.

(0117)

Table 3

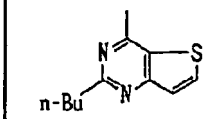
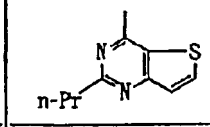
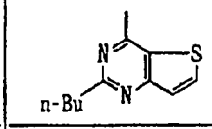
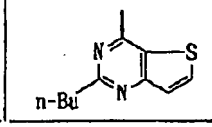


Me = methyl group, Et = ethyl group, n-Pr = n-propyl group, n-Bu = n-butyl group, t-bu = t-butyl group, n-Pe = n-pentyl group, Ac = acetyl group, Ph = phenyl group.

Example	A	R^1	R^2	R^3	R^4	R^5	mp°C
63		H	H	H		H	97-99
64		2-Cl	H	H		H	99-101

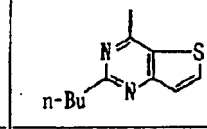
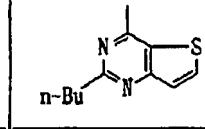
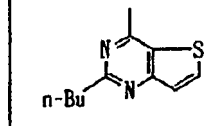
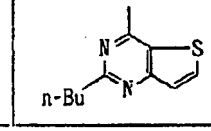
(0118)

Table 3 (continued)

Example	A	R^1	R^2	R^3	R^4	R^5	mp (°C)
65		2-OMe	H	H		H	122-124
66		3-OMe	4-OMe	5-OMe		H	86-88
67		2-Cl	4-Cl	H		H	139-141
68		3-Cl	H	5-OMe		H	74-76

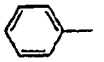
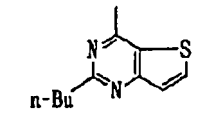
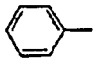
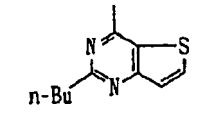
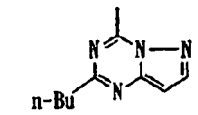
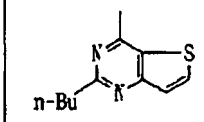
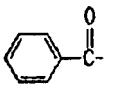
(0119)

Table 3 (continued)

Example	A	R^1	R^2	R^3	R^4	R^5	mp (°C)
69		4-Cl	H	H		H	129-131
70		3-OMe	H	H		H	117-119
71		4-OMe	H	H		H	100-102
72		2-CF_3	H	H		H	155-157

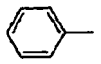
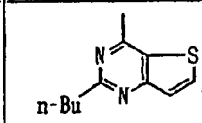
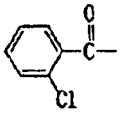
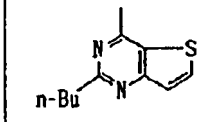
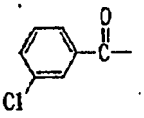
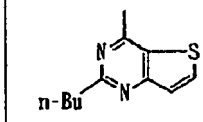
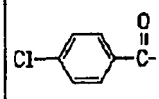
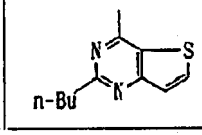
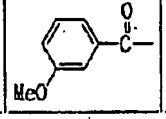
(0120)

Table 3 (continued)

Example	A	R^1	R^2	R^3	R^4	R^5	mp (°C)
73		2-OMe	3-OMe	4-OMe		H	100-102
74		2-SMe	H	H		H	123-125
75		2-OAc	3-OMe	H		H	114-117
76		H	H	H			155-157

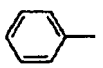
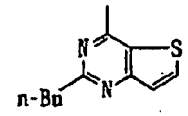
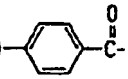
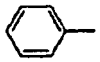
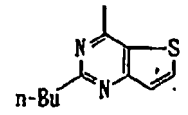
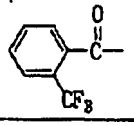
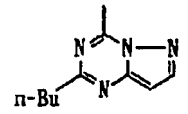
(0121)

Table 3 (continued)

Example	A	R^1	R^2	R^3	R^4	R^5	mp (°C)
77		2-Cl	H	H			111-113
78		3-Cl	H	H			142-144
79		4-Cl	H	H			186-188
80		3-OMe	H	H			134-136

(0122)

Table 3 (continued)

Example	Ⓐ—	R^1	R^2	R^3	R^4	R^5	mp (°C)
81		4-OMe	H	H			140-142
82		2-CF_3	H	H			133-135
83		2-OH	3-OMe	H		H	163-165

(0123)

Examples 84-120

By carrying out the same reaction as in the said Reference Examples and Examples using suitable starting material, it is possible to produce following each compound.

(0124)

Example 84

N-(2-n-butyl thieno[3,2-d]pyrimidine-4-yl)-1-naphthoyl amide.

Example 85

N-(2-n-butyl-9H-purine-6-yl)-1-naphthoyl amide.

Example 86

N-(1-benzyl-6-n-butyl-1H-pyrazolo[3,4-d]pyrimidine-4-yl)-1-naphthoyl amide.

Example 87

N-(8-bromo-2-n-butyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl)-1-naphthoyl amide.

Example 88

N-(2-n-butyl thieno[3,2-d]pyrimidine-4-yl) nicotinamide.

Example 89

N-(2-n-butyl-9H-purine-6-yl) nicotinamide.

Example 90

N-(1-benzyl-6-n-butyl-1H-pyrazolo[3,4-d]pyrimidine-4-yl) nicotinamide.

Example 91

N-(8-bromo-2-n-butyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl) nicotinamide.

Example 92

N-(2-n-butyl thieno[3,2-d]pyrimidine-4-yl)-2-furan carboxamide.

Example 93

N-(2-n-butyl-9H-purine-6-yl)-2-furan carboxamide.

Example 94

N-(1-benzyl-6-n-butyl-1H-pyrazolo[3,4-d]pyrimidine-4-yl)-2-furan carboxamide.

Example 95

N-(8-bromo-2-n-butyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl)-2-furan carboxamide.

Example 96

N-(1-benzyl-6-n-butyl-1H-pyrazolo[3,4-d]pyrimidine-4-yl)-3-chlorobenzamide.

Example 97

N-(2-benzyl-6-n-butyl-2H-pyrazolo[3,4-d]pyrimidine-4-yl)-3-chlorobenzamide.

Example 98

N-(9-benzyl-2-n-butyl-9H-purine-6-yl)-3-chlorobenzamide.

Example 99

N-(7-benzyl-2-n-butyl-7H-purine-6-yl)-3-chlorobenzamide.

Example 100

N-(1-benzyl-6-n-butyl-1H-pyrazolo[3,4-d]pyrimidine-4-yl)-2-methoxy benzamide.

Example 101

N-(2-benzyl-6-n-butyl-2H-pyrazolo[3,4-d]pyrimidine-4-yl)-2-methoxy benzamide.

Example 102

N-(9-benzyl-2-n-butyl-9H-purine-6-yl)-2-methoxy benzamide.

Example 103

N-(7-benzyl-2-n-butyl-7H-purine-6-yl)-2-methoxy benzamide.

Example 104

N-(2-n-butyl-7-phenylpyrazoro [1, 5-a]-1,3,5-triazine-4-yl)-3-chlorobenzamide.

Example 105

N-(2-n-butyl-7-phenylpyrazoro [1, 5-a]-1,3,5-triazine-4-yl)-2-methoxy benzamide.

Example 106

N-(2-n-butyl-8-phenylpyrazoro [1, 5-a]-1,3,5-triazine-4-yl)-3-chlorobenzamide.

Example 107

N-(2-n-butyl-8-phenylpyrazoro [1, 5-a]-1,3,5-triazine-4-yl)-2-methoxy benzamide.

Example 108

N-(2-n-butyl-8-(4-phenylthio phenyl) pyrazolo[1,5-a]-1,3,5-triazine-4-yl)-3-chlorobenzamide.

Example 109

N-(2-n-butyl-8-(4-phenylthio phenyl) pyrazolo[1,5-a]-1,3,5-triazine-4-yl)-2-methoxy benzamide.

Example 110

N-(9-benzyl-2-n-butyl-9H-purine-6-yl)-1-naphthoyl amide.

Example 111

N-(9-benzyl-2-n-butyl-9H-purine=6-yl) nicotinamide.

Example 112

N-(9-benzyl-2-n-butyl-9H-purine = 6-yl)-2-furan carboxamide.

Example 113

N-(7-benzyl-2-n-butyl-7H-purine-6-yl)-1-naphthoyl amide.

Example 114

N-(7-benzyl-2-n-butyl-7H-purine-6-yl) nicotinamide.

Example 115

N-(7-benzyl-2-n-butyl-7H-purine-6-yl)-2-furan carboxamide.

Example 116

N-(2-benzyl-6-n-butyl-2H-pyrazolo[3,4-d]pyrimidine-4-yl)-1-naphthoyl amide.

Example 117

N-(2-benzyl-6-n-butyl-2H-pyrazolo[3,4-d]pyrimidine-4-yl) nicotinamide.

Example 118

N-(2-benzyl-6-n-butyl-2H-pyrazolo[3,4-d]pyrimidine-4-yl)-2-furan carboxamide.

Example 119

N-(8-bromo-2-n-butyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl)-3-chlorobenzamide.

Example 120

N-(8-bromo-2-n-butyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl)-2-methoxy benzamide.

(0125)

Preparation Example 1

Preparation of encapsulated formulation.

Using N-(2-n-butyl thieno[3,2-d]pyrimidine-4-yl)-3,4,5-trimethoxy benzamide as effective ingredient, hard gelatin capsules (1000 capsules) containing 250 mg per 1 capsule was prepared by the following formulation.

(00126)

Effective ingredient compound	250 g
Crystalline cellulose (Pharmacopeia of Japan product)	30 g
Corn starch (Pharmacopeia of Japan product)	17 g
Talc (Pharmacopeia of Japan product)	2 g
Magnesium stearate (Pharmacopeia of Japan product)	1 g

In other words, each component was made into fine powder in accordance with aforesaid formulation, it was sufficiently mixed to form a uniform mixture, thereafter this was packed in gelatin capsule for the oral administration having desired dimension and the target encapsulated formulation was prepared.

(0127)

Preparation Example 2

Preparation of tablet.

Using N-(2-n-butyl pyrazolo[1,5-a]-1,3,5-triazine-4-yl)-2,4-dichlorobenzamide as effective ingredient compound, tablets (2000 tablets) containing 300 mg per tablet was prepared by the following formulation.

(00128)

Effective ingredient compound.	600 g
Lactose (Pharmacopeia of Japan product)	67 g
Corn starch (Pharmacopeia of Japan product)	33 g
Carboxymethylcellulose calcium (Pharmacopeia of Japan product)	25 g
Methyl cellulose (Pharmacopeia of Japan product)	12 g
Magnesium stearate (Pharmacopeia of Japan product)	3 g

In other words, effective ingredient compound, lactose, corn starch and carboxymethylcellulose calcium were mixed thoroughly according to the aforesaid formulation, and the mixture was granulated using methyl cellulose aqueous solution and was passed through sieve of 24 mesh, and this was mixed with magnesium stearate and was pressed to tablet, and the target tablet was prepared.

(0129)

Pharmacological Test Example 1

Spraque Dawley strain male rats (6-9 weeks old, 200-250 g) were slaughtered by cervical spine dislocation, and thoracic aorta was extracted promptly, and surrounding connective tissues were peeled off. Next, the aorta was cut into 5-7 rings, each was sliced open longitudinally, and thereafter intravascular cavity was abraded using a washed swab thereby eliminating endothelial cells in order to eliminate the effect of cNOS present in vascular endothelial cells, and sample was prepared.

(0130)

The aforesaid sample was introduced into Krebs-Henseleit liquid (NaCl 118.3 mM, KCl 4.7 mM, CaCl2 2.5 mM, KH2PO4 1.2 mM, $MgSO_4$ 1.2 mM, NaHCO3 25.0 mM and glucose 11.1 mM) wherein dimethylsulfoxide solution of effective ingredient compound of this invention (test compound) which was prepared in 30 μM concentration was added and L-arginine was further added so as to become 400 μM concentration, and the mixture was incubated at 37°C for 30 minutes. Continuing lipopolysaccharide (LPS) was added by 1000 ng/ml concentration, and it

was incubated at 37°C for 24 hours (experimental group using test compound, group of this invention).

(0131)

Next, supernatant was sampled on 96-well plate, and NO2 was coloured with Griess liquid according to NO2 measurement method described in literature (New Biochemistry Experiment chair 10, blood vessel, endothelium and smooth muscle, 135 pages, Jpn Biochem Soc Eds, Tokyo Kagaku Dojin, 1993) and it was measured using Biokinetics Reader (EL-340 model, made by BIO-TEK Instruments company), and accumulated NO2 amount was calculated.

(0132)

Moreover, the sample of blood vessel piece was dissolved in 1N sodium hydroxide aqueous solution, and it was coloured with Bio-Rad DC protein assay kit (made by Bio-Rad Laboratories Co) and it was measured with spectrophotometer (made by HITACHI Co, U-3000 model), and protein content was calculated. Moreover, from these values, the quantity of NO2 formed per protein 1 mg was determined.

(0133)

On the other hand, the same test was carried out for the control group with the addition of dimethylsulfoxide instead of the test compound for the negative control group without even the addition of LPS.

(0134)

The iNOS induction inhibition rate was determined according to the following equation from NO2 quantity formed per protein 1 mg in each group obtained as above.

(0135)

Inhibition rate (%) ={1- [(this invention group value) - (negative control group value)] / [control group value) - (negative control group value)]} x 100

The obtained results are shown in the Table 6.

(0136)

Table 4

Test Compound (Example No.)	Inhibition rate (%)
1	81.5
9	53.9
15	60.0
16	36.8
33	37.6
64	51.3
67	57.4
70	55.2

(0137)

From Table 4, it is clear that effective ingredient compound of this invention inhibits induction of iNOS by LPS.

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